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Distribution and Prevalence of the Asian Fish Tapeworm, *Bothriocephalus acheilognathi*, in the Colorado River and Tributaries, Grand Canyon, Arizona, Including Two New Host Records

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ABSTRACT: The Asian fish tapeworm, *Bothriocephalus acheilognathi*, has invaded the lower Little Colorado River (LCR), a tributary of the Colorado River, where it infects humpback chub (*Gila cypha*), speckled dace (*Rhinichthys osculus*), and fathead minnow (*Pimephales promelas*). This study examined the distribution of *B. acheilognathi* in the Colorado River and tributaries in Grand Canyon. In 1994, 22.5% of humpback chub, 10.3% of plains killifish (*Fundulus zebrinus*), 3.8% of speckled dace, and 2.2% of fathead minnow were infected. In 1995, 2.4% of fathead minnow and 1.4% of speckled dace were infected. Humpback chub, an endangered species, and plains killifish are new host records for this parasite. Nearly all (66.7 to 100%) infected fish were captured in areas near the LCR and were probably the result of infected fish emigrating from that tributary. However, 4 infected fish (1 plains killifish, 1 speckled dace, and 2 fathead minnows) were caught 92.8 to 202.1 km downstream from the LCR. Another speckled dace was caught in the lower section of Kanab Creek, a warm tributary, indicating a potential expansion of the parasite's range. Infection of humpback chub by *B. acheilognathi* is of concern due to the endangered status of this fish. Because *B. acheilognathi* requires high water temperature for completion of its life cycle, this species is largely confined to the LCR by the cold water of the mainstem Colorado River. The potential effects of plans to seasonally warm the Colorado River on *B. acheilognathi* are discussed.

KEY WORDS: *Bothriocephalus acheilognathi*, Colorado River, humpback chub, speckled dace, fathead minnow, plains killifish, distribution, prevalence.

The closure of Glen Canyon Dam in 1963 turned the Colorado River in Grand Canyon from a seasonally warm and muddy river into a typically clear and constantly cold one due to hypolimnetic discharge from Lake Powell. The drastic changes in the riverine environment, particularly water temperature and turbidity, caused by the closure of Glen Canyon Dam, have had a severe negative impact on the native fishes in Grand Canyon (Minckley, 1991). Of the original 8 endemic fishes in Grand Canyon, reproducing populations of only 4 remain, one of which is endangered. Lost to this reach of the Colorado River are the Colorado squawfish (*Ptychocheilus lucius*), bonytail chub (*Gila elegans*), and roundtail chub (*G. robusta*), and the razorback sucker (*Xyrauchen texanus*) is extremely rare and probably not reproducing. Remaining are humpback chub (*G. cypha*; federally endangered), flannelmouth sucker (*Catostomus latipinnis*; category II), bluehead sucker (*Catostomus discobolus*), and speckled dace (*Rhinichthys osculus*). Reproduction of these fish is now largely restricted to a few perennial tributaries (AGFD, 1996); however, backwaters of the mainstem Colorado River are important rearing areas for larval and juvenile native and exotic fish (Holden, 1978; Valdez and Clemmer, 1982; Carter et al., 1985; AGFD, 1996).

Recently, a management proposal (Bureau of Reclamation, 1995) suggested the installation of a multilevel intake structure (MLIS) in Glen Canyon Dam to increase downstream water temperatures seasonally and improve conditions for native fish. Changing from hypolimnetic releases to epilimnetic releases in the spring may provide sufficient temperature elevation for increased mainstem reproduction and survival and growth of native young-of-the-year (YOY) fish. However, an important consideration of warming the river is the potential for an increase in the incidence of fish parasites and diseases.

The Asian fish tapeworm, *Bothriocephalus acheilognathi*, a pseudophyllidean cestode, was originally described from *Acheilognathus rhombea* in Japan (Yamaguti, 1934). It has spread to Europe, Russia, and North America with introductions of grass carp (*Ctenopharyngodon idella*) in the early 1970's (Hoffman and Shubert, 1984). *Bothriocephalus acheilognathi* is now well established in golden shiner (*Notemigonus crysoleucas*), red shiner (*Cyprinella lutrensis*),

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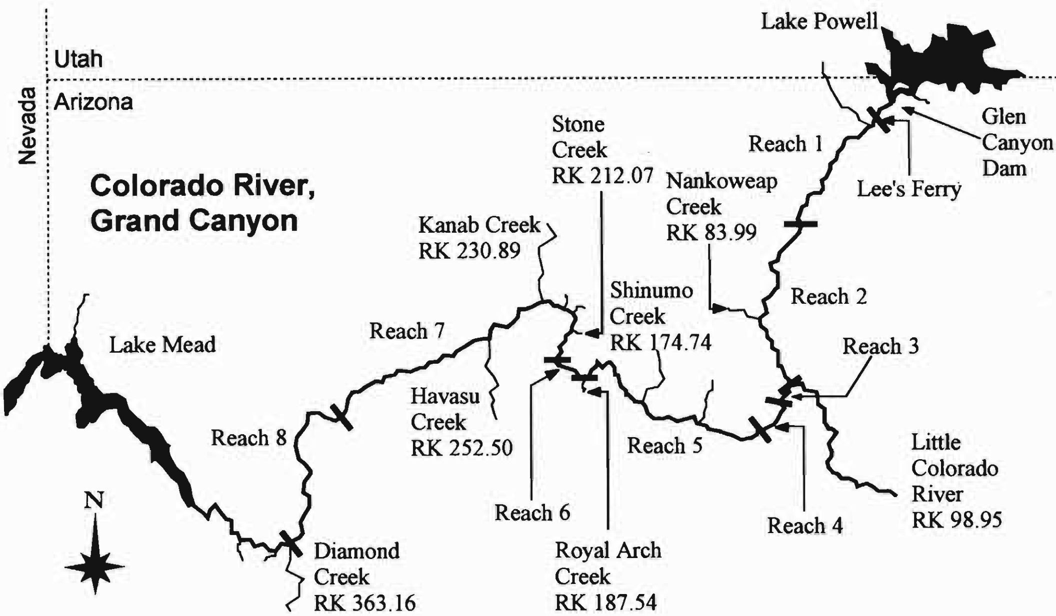

Figure 1. Boundaries (bars) of 8 designated fish sampling reaches and location of sampled tributaries of the Colorado River, Grand Canyon, Arizona. River kilometer (RK) is distance downstream from Lee's Ferry. Colorado River reach boundaries are as follows: Reach 1—Lee's Ferry (RK 0) to Shinumo Wash (RK 47.17); Reach 2—Shinumo Wash to Little Colorado River (RK 99.0); Reach 3—LCR to Lava Chuar Rapid (RK 105.44); Reach 4—Lava Chuar Rapid to Hance Rapid (RK 123.47); Reach 5—Hance Rapid to Elve's Chasm (Royal Arch Creek, RK 187.54); Reach 6—Elve's Chasm to Forster Rapid (RK 197.68); Reach 7—Forster Rapid to Hell's Hollow (RK 293.78); Reach 8—Hell's Hollow to Diamond Creek (RK 363.16).

fathead minnow (*Pimephales promelas*), grass carp, and mosquitofish (*Gambusia affinis*) in the mid-south and southeastern United States (Hoffman and Schubert, 1984; Riggs and Esch, 1987). Heckmann et al. (1987) found *B. acheilognathi* in speckled dace (*Rhinichthys osculus*), red shiner, the endangered woundfin (*Plagopterus argentissimus*), Virgin River chub (*G. robusta seminuda*), and Virgin spinedace (*Lepidomeda mollispinis*) from the Virgin River, Utah, Nevada, and Arizona. Heckmann et al. (1993) found *B. acheilognathi* elsewhere in Nevada: in red shiner in the Muddy River, roundtail chub from the Moapa Power Plant cooling pond, and golden shiner from bait shops around Las Vegas. Font and Tate (1994) have also reported *B. acheilognathi* from native Hawaiian freshwater fish.

Cyclopoid copepods are the intermediate hosts of *B. acheilognathi* (Marcogliese and Esch, 1989a), and the definitive hosts are a broad range of fish, particularly cyprinids (Hoffman and Schubert, 1984). Temperatures in excess of 20°C are required for maturation of this cestode (Granath and Esch, 1983a). Currently, *B. acheilognathi* appears to be confined to the Little Colorado River (LCR), probably by cold mainstem water temperatures that do not reach 20°C (Stanford and Ward, 1991). Temperatures in many of the other tributaries throughout Grand Canyon are similar to those in the LCR (AGFD, 1996) and thus should be capable of colonization by *B. acheilognathi*. This study examined the present distribution and prevalence of *B. acheilognathi* in native and exotic fish of the Colorado River, Grand Canyon, and its tributaries.

Materials and Methods

Native and exotic fish were collected in 1994 and 1995. In 1994, as part of a diet study of small (<150 mm) fish, we attempted to collect 5 fish from each of 2 size classes, ≤30 mm and >30 mm total length, from each of the 8 mainstem Colorado River reaches (Figure 1) during each of 3 river trips. These species included: humpback chub, speckled dace, flannelmouth sucker, bluehead sucker, rainbow trout (*Oncorhynchus mykiss*), fathead minnow, and plains killifish (*Fundulus*

Table 1. Prevalence of *Bothriocephalus acheilognathi* in humpback chub and plains killifish collected from the Colorado River and tributaries, Grand Canyon, Arizona, 1994. Dashes indicate that no collections were attempted at that location.

	Humpback chub			Plains killifish		
Reach/Tributary	*N*	Number infected	Percent infected	*N*	Number infected	Percent infected
1	0	0	0.0	0	0	0.0
2	4	1	25.0	1	0	0.0
Nankoweap Creek	—	—	—	—	—	—
Little Colorado River	—	—	—	—	—	—
3	39	16	41.0	9	1	11.1
4	58	10	17.2	12	1	8.3
5	0	0	0.0	1	0	0.0
Shinumo Creek	—	—	—	—	—	—
Royal Arch Creek	—	—	—	—	—	—
6	9	0	0.0	5	1	20.0
7	7	0	0.0	1	0	0.0
Stone Creek	—	—	—	—	—	—
Kanab Creek	—	—	—	—	—	—
Havasu Creek	—	—	—	—	—	—
8	3	0	0.0	0	0	0.0
Total	120	27	22.5	29	3	10.3

zebrinus). In 1995, we further examined the distribution of this parasite by attempting to collect 5 speckled dace and 5 fathead minnows from each of the 8 reaches and 7 tributaries on each of 3 river trips. We were not permitted to collect humpback chub in 1995 due to its endangered status. Fish were collected using seines, hoop nets, dip nets, minnow traps, and electrofishing. Total length (TL; mm) and weight (g) of fish and date and location of capture (tributary or river kilometer (RK) downstream from Lee's Ferry) were recorded. Fish were then preserved in either 70% ethanol or 10% formalin, as field examination was not practical. In the laboratory, fish were examined to determine the presence or absence of Asian fish tapeworms in each fish. A representative specimen of *B. acheilognathi* has been deposited in the U.S. National Parasite Collection, Beltsville, Maryland (USPNC Coll. No. 86818).

Results

A total of 1,902 fish representing 7 species in 4 families was examined for *B. acheilognathi* in this study. These fish included all 4 remaining native species and 3 common exotic species. Species sampled included: humpback chub, speckled dace, and fathead minnow (Cyprinidae); plains killifish (Cyprinodontidae); bluehead and flannelmouth suckers (Catostomidae); and rainbow trout (Salmonidae).

In 1994, 1,669 fish were sampled from the mainstem Colorado River. Twenty-seven of 120 (22.5%) humpback chub (12 to 110 mm TL; $\bar{x}$ = 36.2 mm), 7 of 185 (3.8%) speckled dace (12 to 132 mm TL; $\bar{x}$ = 35.7 mm), 5 of 234 (2.2%) fathead minnows (12 to 78 mm TL; $\bar{x}$ = 35.1 mm), and 3 of 29 (10.3%) plains killifish (21 to 57 mm TL; $\bar{x}$ = 35.1 mm) were infected with *B. acheilognathi* (Tables 1, 2, and 3). None of 329 flannelmouth suckers (15 to 98 mm TL; $\bar{x}$ = 34.2 mm), 562 bluehead suckers (12 to 106 mm TL; $\bar{x}$ = 29.0), or 210 rainbow trout (22 to 416 mm TL; $\bar{x}$ = 267.4 mm) were infected.

In 1995, 148 speckled dace and 85 fathead minnows were examined from the Colorado River and 7 tributaries in Grand Canyon. Two of 85 (2.4%) fathead minnows and 2 of 148 (1.4%) speckled dace were infected with *B. acheilognathi*.

In both 1994 and 1995, the majority of the fish infected with *B. acheilognathi* were captured in the reach directly above (Reach 2) and 2 reaches directly below (Reaches 3 and 4) the confluence of the Little Colorado and Colorado rivers. Of the infected fish, all of the 27 humpback chub, 7 of 9 (77.8%) speckled dace, 5 of 7 (71.4%) fathead minnow, and 2 of 3 (66.7%) plains killifish were captured in this area. Of greater interest are the fish captured outside of this area. One infected fathead minnow was captured at RK 265.2 and another at RK 301.1. One infected plains killifish was captured at RK 191.8. An infected speckled dace was captured in Kanab Creek (RK 230.9) and another in a backwater at RK 266.6. No infected fish were

Table 2. Prevalence of *Bothriocephalus acheilognathi* in speckled dace collected from the Colorado River and tributaries, Grand Canyon, Arizona, 1994 and 1995. Dashes indicate that no collections were attempted at that location.

	1994			1995		
Reach/Tributary	*N*	Number infected	Percent infected	*N*	Number infected	Percent infected
1	7	0	0.0	0	0	0.0
2	18	0	0.0	3	0	0.0
Nankoweap Creek	—	—	—	6	0	0.0
Little Colorado River	—	—	—	11	1	9.1
3	16	2	12.5	13	0	0.0
4	17	4	23.5	15	0	0.0
5	0	0	0.0	1	0	0.0
Shinumo Creek	—	—	—	25	0	0.0
Royal Arch Creek	—	—	—	7	0	0.0
6	17	0	0.0	5	0	0.0
7	32	1	3.1	14	0	0.0
Stone Creek	—	—	—	3	0	0.0
Kanab Creek	—	—	—	4	1	25.0
Havasu Creek	—	—	—	24	0	0.0
8	78	0	0.0	17	0	0.0
Total	185	7	3.8	148	2	1.4

captured in Nankoweap, Shinumo, Royal Arch, Stone, or Havasu creeks.

Discussion

Bothriocephalus acheilognathi was found in 4 of 7 species of fish examined from the Colorado River, Grand Canyon: 3 cyprinids (humpback chub, speckled dace, and fathead minnow) and 1 cyprinodontid (plains killifish). The Colorado and lower Little Colorado rivers are new localities for this parasite, which likely invaded via infected nonnative fish species or copepods from the upper LCR. Although *B. acheilognathi* has not been documented in the upper LCR, common carp, fathead minnow, and plains killifish are all potential hosts of this parasite and are

Table 3. Prevalence of *Bothriocephalus acheilognathi* in fathead minnow collected from the Colorado River and tributaries, Grand Canyon, Arizona, 1994 and 1995. Dashes indicate that no collections were attempted at that location.

	1994			1995		
Reach/Tributary	*N*	Number infected	Percent infected	*N*	Number infected	Percent infected
1	0	0	0.0	0	0	0.0
2	17	0	0.0	3	1	33.3
Nankoweap Creek	—	—	—	0	0	0.0
Little Colorado River	—	—	—	8	0	0.0
3	78	0	0.0	16	0	0.0
4	36	3	8.3	18	1	6.3
5	0	0	0.0	0	0	0.0
Shinumo Creek	—	—	—	0	0	0.0
Royal Arch Creek	—	—	—	0	0	0.0
6	29	0	0.0	13	0	0.0
7	42	1	2.4	15	0	0.0
Stone Creek	—	—	—	0	0	0.0
Kanab Creek	—	—	—	7	0	0.0
Havasu Creek	—	—	—	0	0	0.0
8	32	1	3.1	5	0	0.0
Total	234	5	2.2	85	2	2.4

common upstream, in the perennial headwaters of the LCR in the White Mountains area of eastern Arizona. Although the middle portion of the LCR is ephemeral (from Lyman Lake to 21 km above the confluence with the Colorado River), infected fish or copepods may easily have been flushed downstream into the lower LCR during floods. Although Heckmann et al. (1987) found *B. acheilognathi* in speckled dace at Beaver Dam Wash, Virgin River, Arizona, it is highly unlikely that any of these fish moved down to Lake Mead, then upstream over 400 km and through many large rapids to the LCR. The paucity of infected fish in the lower part of the canyon and the absence of infected fish from the upper canyon, above the LCR, casts doubt on invasion of this area via migration up or down the Colorado River.

This is the first report in the refereed literature of *B. acheilognathi* in the endangered humpback chub. *Bothriocephalus acheilognathi* has been reported in humpback chub and speckled dace in the LCR in 1990 in an Arizona Game and Fish Department agency report (Clarkson and Robinson, 1993). This cestode was first discovered in the Grand Canyon in May 1990 in humpback chub from the LCR (C.O. Minckley, U.S. Fish and Wildlife Service, pers. comm.). Kaeding and Zimmermann (1983) examined 26 humpback chub for pathogens from the LCR and Colorado River from 1979 to 1981. They reported 13 bacteria, 6 protozoans, 1 fungus, and the parasitic copepod *Lernaea cyprinacea*, but not *B. acheilognathi*. Heckmann et al. (1987) and Heckmann et al. (1993) reported *B. acheilognathi* in the closely related roundtail chub. Infection of humpback chub by *B. acheilognathi* is expected since copepods were found in 7.2% of the humpback chub stomachs collected in 1994 (AGFD, 1996).

The occurrence of *B. acheilognathi* in 10.3% of the plains killifish examined is also the first report of this species as a host for this parasite. However, mosquitofish, another cyprinodontid, is also susceptible to this parasite (Granath and Esch, 1983b; Riggs and Esch, 1987; Marcogliese and Esch, 1989b). None of the 29 plains killifish examined from 1994 contained copepods in their stomachs (AGFD, 1996). Plains killifish is a surface feeder and is omnivorous, with insects and aquatic invertebrates being dominant food items (Shute and Allen, 1980), but they may also consume benthic material (Simon, 1946). Although copepods do not appear to be a dominant food item for plains killifish, infection with *B. acheilognathi* indicates that copepods are occasionally ingested.

Neither flannelmouth sucker, bluehead sucker, nor rainbow trout contained *B. acheilognathi*. Copepods were ingested by both species of suckers, more so by flannelmouth suckers (AGFD, 1996). Presumably, all species that ingested copepods were exposed to this parasite, since they were collected from the same sites as infected species. Therefore, since both sucker species contained copepods in their stomachs, it appears that they are not susceptible to infection by the Asian fish tapeworm. This result supports Heckmann et al. (1987), who found no *B. acheilognathi* in the 3 flannelmouth suckers they examined. No copepods were found in rainbow trout stomachs. Although most of the trout were caught upstream from the LCR, to our knowledge, *B. acheilognathi* has never been reported in salmonids.

Bothriocephalus acheilognathi was found in fish collected throughout the entire mainstream Colorado River and 2 tributaries from RK 97.9 to RK 301.1. However, the majority of the fish that were infected by *B. acheilognathi* were captured in reaches directly above and below the confluence of the Little Colorado and Colorado rivers (Reaches 2, 3, and 4) and were probably the result of fish emigrating from the LCR. The fish caught upstream from the LCR were captured in a backwater only 1.1 km from the mouth of the LCR and did not need to negotiate any rapids to get there. Therefore, it appears that *B. acheilognathi* is currently only able to complete its life cycle within the LCR.

Five infected fish were captured outside of the LCR and nearby reaches, at least 92.8 km and as far as 202.1 km downstream from the LCR. Of particular concern is the speckled dace captured in Kanab Creek. Water temperatures of Kanab Creek are warm enough to allow for reproduction by *B. acheilognathi*, with mean temperatures exceeding 20°C from May through August and reaching as high as 34°C (Otis, 1994; AGFD, 1996). Whether this occurrence of an infected speckled dace is indicative of a separate, reproducing population of *B. acheilognathi* or simply an infected fish that had emigrated downstream from the LCR is not clear. This fish was caught in the lower section (<500 m from the mouth) of Kanab Creek, so either alternative

is possible. In any event, since speckled dace and fathead minnow are resident and humpback chub are occasionally found in Kanab Creek (AGFD, 1996), the potential certainly exists for this parasite to become established in this tributary. Therefore, although the cold water temperature of the mainstem Colorado River seems to be limiting the distribution of this parasite, there is an indication that it may have colonized Kanab Creek. Further examination of the potential colonization of Kanab Creek by *B. acheilognathi* is warranted and planned.

Three of the 4 components for successful invasion by *B. acheilognathi* are present in the mainstem of the Colorado River, Grand Canyon. First, definitive hosts (native and exotic cyprinid fishes) are present throughout the river. Secondly, the intermediate host, cyclopoid copepods, are abundant in the mainstem of the Colorado River and are ingested by native and exotic fishes (AGFD, 1996). Thirdly, *B. acheilognathi* is present in the lower reaches of the LCR (Clarkson and Robinson, 1993). The fourth and apparently limiting factor is water temperature. Although the temperature in the mainstem of the Colorado River is currently too cold for the parasite to disperse throughout the entire Grand Canyon, the proposed MLIS could increase the water temperature in the mainstem by 3 to 10°C (Bureau of Reclamation, 1995). This could cause water in the mainstem to reach the minimum temperature required for *B. acheilognathi* to complete its life cycle. Even if main channel temperatures do not reach 20°C, backwater temperatures will certainly exceed 20°C and may permit *B. acheilognathi* to complete its life cycle in these habitats or improve the chances of its colonizing other tributaries.

The major factor affecting egg maturation, coracidium motility, growth, development of adult worms, and ultimately the size and composition of *B. acheilognathi* populations is water temperature. Granath and Esch (1983a) found that growth and development of this parasite was stimulated by temperatures above 25°C and that temperatures of 25 to 30°C maximized egg maturation, hatching, and coracidium motility. At temperatures outside that range, these activities were depressed. Temperatures exceeding 35°C caused a decrease in recruitment of this parasite in mosquitofish (Granath and Esch, 1983b). Water temperature in the LCR is suitable for *B. acheilognathi*, exceeding 20°C from May through September in 1993 and reaching as high as 26.1°C (Gorman, 1994). Conversely, temperatures in the main channel Colorado River are unsuitable for this parasite, reaching only 18.4°C from 1991 to 1994 (AGFD, 1996). Maximum backwater temperatures reached 28.0°C in shallow areas, suitable for *B. acheilognathi* to complete its life cycle. However, the water level in the Colorado River, Grand Canyon, fluctuates daily with the demand for electric power. This dynamic nature causes inundation and desiccation of backwaters, prevents these temperatures from being stable, and flushes zooplankton from the backwaters. Mean backwater temperatures never exceeded 20°C during 4 yr of study (AGFD, 1996). However, construction of an MLIS or implementation of steady flows, another suggested mitigation measure (U.S. Fish and Wildlife Service, 1994), will likely cause mean backwater temperatures to increase above 20°C and may regularly approach 25°C, aiding the completion of the life cycle of *B. acheilognathi*. Copepods inhabit backwaters, which are important rearing areas for larval and juvenile native and exotic fishes (AGFD, 1996). These fish are planktivorous and regularly ingest copepods (AGFD, 1996). Therefore, warming of the river may permit *B. acheilognathi* to expand its range beyond the LCR.

The infection rates for speckled dace (1.4 to 3.8%) and fathead minnow (2.2 to 2.4%) in this study were relatively low. Only 1.4% of the speckled dace and none of the fathead minnows sampled from 1994 contained copepods in their stomachs (AGFD, 1996). The fact that fathead minnows were infected with *B. acheilognathi* indicates that copepods are ingested. Clarkson and Robinson (1993) also found low prevalence (0.4%) in speckled dace from the LCR captured in 1991 but higher prevalence (17.0%) in 1992. Heckmann et al. (1987) found 17% of 107 speckled dace were infected from Beaver Dam Wash, Virgin River, Arizona. Riggs and Esch (1987) found prevalence of *B. acheilognathi* in fathead minnows in Belews Lake, North Carolina, to range from approximately 15 to 95%, depending on season and site. The low prevalence of *B. acheilognathi* in speckled dace and fathead minnow in our study may be due to the dynamic nature of the LCR. The base discharge of the LCR, approximately 5.6 m^3/s, comes from a series of springs approximately 21 km from the mouth (Minckley, 1991). However, flooding

is common in spring (rain and snow melt) and in late summer (monsoonal rains) and can exceed 850 m^3/s. Differences in the frequency and severity of these floods may have dramatic effects on copepod populations, which would then affect *B. acheilognathi* populations, as noted by Marcogliese and Esch (1989b). These low prevalences of *B. acheilognathi* in speckled dace and fathead minnow also reflect our inclusion of samples from areas where the parasite was not found.

The infection of humpback chub by *B. acheilognathi* (rates as high as 41% in Reach 3 and an overall infection rate of 22.5% in 1994) is of concern, considering the endangered status of this fish. Clarkson and Robinson (1993) reported infection rates in juvenile humpback chub in the LCR as high as 78.9% in 1990 and 77.8% in 1992 and as low as 12.4% in 1991 and 0% in 1989 (1989 may have been preinvasion). Valdez and Ryel (1995) reported finding *B. acheilognathi* in 3.6% of 168 adult (>250 mm) humpback chub. However, the prevalence of *B. acheilognathi* in these fish may have actually been higher since their data were from stomach contents obtained by flushing the gastrointestinal (GI) tract and may not have dislodged parasites from all infected fish. High intensity infections can lead to mortality by blockage of the GI tract, intestinal perforation, and/or destruction of the intestinal mucosa, killing the fish (Hoffman, 1980; Schäperclaus, 1986). The humpback chub in and around the Little Colorado River comprise the largest remaining population of this species (Maddux et al., 1993). Douglas and Marsh (1996) used various models to estimate population size of humpback chub (>150 mm TL) in Grand Canyon, which ranged from 4,508 to 10,444 fish. This small population size, high infection rates, and potential for mortality do not bode well for this endangered fish.

The proposed MLIS may increase water temperatures of the mainstem Colorado River 3 to 10°C (Bureau of Reclamation, 1995). This increase in mainstem water temperature may be high enough to allow *B. acheilognathi* to become established in other tributaries and possibly the mainstem of the Colorado River in Grand Canyon. This is a more likely scenario in streams further downstream from Glen Canyon Dam where the water is warmer. Increasing mainstem water temperature may initially increase growth and survival of YOY native fish. However, it may also prove to be detrimental to these fish in the long run, due to an increase in the prevalence of *B. acheilognathi* and/or other parasites and diseases.

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